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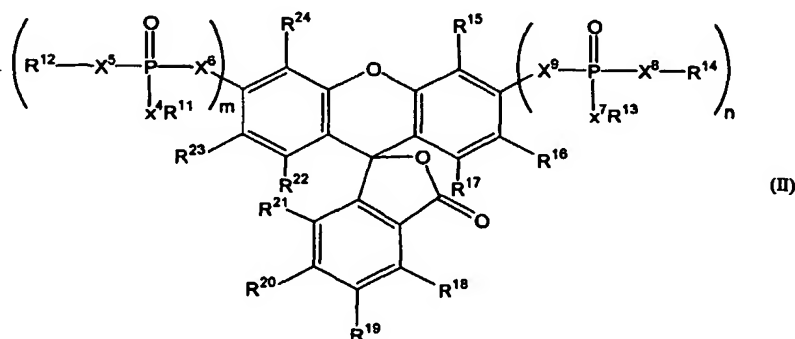
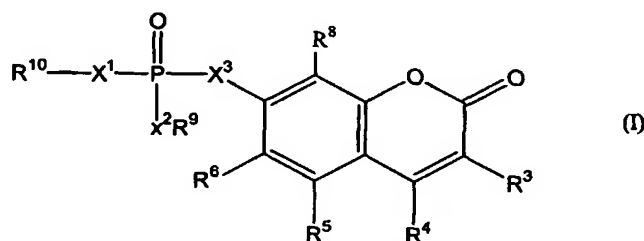
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[Continued on next page]

(54) Title: **FLUORESCENT SUBSTRATES FOR DETECTING ORGANOPHOSPHATASE ENZYME ACTIVITY**



(57) Abstract: Disclosed are compounds of the formula (I): wherein R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, and R<sup>10</sup> are selected from the group consisting of H and groups or atoms other than H, and R<sup>6</sup> and R<sup>8</sup> are halo or hydrogen; X<sup>1</sup>, X<sup>2</sup>, and X<sup>3</sup> are independently O or S; provided that R<sup>9</sup> and R<sup>10</sup> are not simultaneously H, when all of X<sup>1</sup>, X<sup>2</sup>, and X<sup>3</sup> are O; and of the formula (II) wherein R<sup>11</sup>-R<sup>14</sup> are selected from the group consisting of H and groups or atoms other than H; X<sup>4</sup>-X<sup>9</sup> are independently O or S; n and m are 0 or 1 but m and n cannot be 0 simultaneously; R<sup>15</sup>-R<sup>24</sup> can be H or any substituent so long as the compound of formula II upon hydrolysis provides a fluorescent compound. These compounds are useful as substrates with high specificity for organophosphatase particularly human paraoxonase and bacterial organophosphorus hydrolase. Also disclosed is a method for detecting and/or measuring the paraoxonase activity in a fluid comprising contacting

the fluid with a fluorescent substrate and measuring the fluorescence of the fluorescent product formed.



**(88) Date of publication of the international search report:**  
13 January 2005

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US2004/007897

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C12Q1/42 C07F9/09

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12Q C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/092803 A (HORNE IRENE ; RUSSELL ROBYN (AU); OAKESHOTT JOHN (AU); SUTHERLAND TARA) 21 November 2002 (2002-11-21)  page 6, lines 7-31; page 7, lines 3-6; page 7, line 21 - page 8, line 8; pages 33-35; claims 33-35	1-13, 16, 18, 21, 22, 25, 28-30, 33, 34, 37, 40
X	EP 0 949 266 A (BIOSYNTH AG) 13 October 1999 (1999-10-13)  page 2, paragraphs '0006!-'0009!; page 3, paragraph '0015!; page 4, lines 1-16; examples 1 and 3; claims 4, 5 and 17  -/--	1-7, 10-13, 18, 30, 37

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*G\* document member of the same patent family

Date of the actual completion of the international search

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## INTERNATIONAL SEARCH REPORT

 Int 1al Application No  
 PCT/US2004/007897

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOVACS PETER ET AL: "Fluorimetric analysis of phospholipase activity in <i>Tetrahymena pyriformis</i> GL" BIOSCIENCE REPORTS, vol. 19, no. 2, April 1999 (1999-04), pages 81-87, XP009034219 ISSN: 0144-8463 page 82, "Materials and Methods", "In vitro Experiments"; page 83, "In vivo Experiments"	1-7, 10-13, 18,30,37
X	WO 03/020984 A (AMERSHAM BIOSCIENCES CORP) 13 March 2003 (2003-03-13) examples 1 and 2	1-3
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X	US 5 011 964 A (MYNARCIK DENNIS C ET AL) 30 April 1991 (1991-04-30) example VIII	1-3
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In Application No  
PCT/US2004/007897

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GEE KYLE R ET AL: "Fluorogenic substrates based on fluorinated umbelliferones for continuous assays of phosphatases and beta-galactosidases" ANALYTICAL BIOCHEMISTRY, vol. 273, no. 1, 15 August 1999 (1999-08-15), pages 41-48, XP002289576 ISSN: 0003-2697 Page 42, "Materials and Methods"; page 43, figure 1, compounds 2a-2e	1-7,9-14
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X	HUANG Z ET AL: "3,6-FLUORESCEIN DIPHOSPHATE: A SENSITIVE FLUOROGENIC AND CHROMOGENIC SUBSTRATE FOR PROTEIN TYROSINE PHOSPHATASES" JOURNAL OF BIOMOLECULAR SCREENING, LARCHMONT, NY, US, vol. 4, no. 6, December 1999 (1999-12), pages 327-334, XP001041328 ISSN: 1087-0571 page 328, right-hand column; page 329, figure 2; page 331, left-hand column	43-48, 51,54
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Y	page 1395, scheme; page 1396, table and figure	50,52,53
X	US 5 830 666 A (FUJITA SATOSHI ET AL) 3 November 1998 (1998-11-03) column 1, lines 61-67; column 4, line 30 - column 5, line 30; column 6, line 21 - column 8, line 56; column 12, line 65 - column 17, line 36	43-48, 51,54-56
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## INTERNATIONAL SEARCH REPORT

In            national Application No  
PCT/US2004/007897

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZAIKOVA TATIANA O ET AL: "Synthesis of fluorogenic substrates for continuous assay of phosphatidylinositol-specific phospholipase C" BIOCONJUGATE CHEMISTRY, vol. 12, no. 2, March 2001 (2001-03), pages 307-313, XP002301312 ISSN: 1043-1802	43-49,51
Y	page 308, compound 1; page 310, scheme 3; page 311, compound 17 -----	50,52,53
X	WANG Q ET AL: "Novel caged fluorescein diphosphates as photoactivatable substrates for protein tyrosine phosphatases" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1601, no. 1, 19 November 2002 (2002-11-19), pages 19-28, XP004392051 page 21, figure 1 -----	43-49,51

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2004/007897

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of Item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the International application and necessary to the claimed invention, the International search was carried out on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



In written format



in computer readable form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in computer readable form



furnished subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2004/007897

### Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-42 (entirely)

Compound of formula (I) and method for detecting or measuring the presence of organophosphatase enzyme in a fluid or immobilized on a solid support.

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2. claims: 43-56 (entirely)

Compound of formula (II) and method for detecting or measuring the presence of organophosphatase enzyme in a fluid or immobilized on a solid support.

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# INTERNATIONAL SEARCH REPORT

1st Application No  
PCT/US2004/007897

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